Synchrotron X-ray Footprinting of Apomyoglobin Equilibrium Folding

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In order to establish protein folding analysis through synchrotron footprinting experiments, apomyoglobin has been selected as a model since its cooperative folding transition has been well characterized. Our synchrotron protein footprinting method is based on quantitative analysis of modification of amino acid side chains as a function of solvent accessibility. Apomyoglobin consists of 8 helices that all contain modifiable amino acids (see subprojects 8 & 9) that serve as structural probes for this analysis. Our mass spectrometric based analysis examines the change in modification of selected amino acids in each helix as the protein makes its transition to an unfolded state. For this purpose, solutions of apomyoglobin in a series of denaturant concentrations (such as urea) are exposed to the synchrotron beam. The protein is then digested and the mixture is analyzed by HPLC and electrospray mass spectrometry. This procedure revealed an unfolding mid-point of 3M urea for peptide 1-16:GLSDGEWQQVLNVWGK of A-helix that agrees quite well with fluorescence studies.